DECREASING LACTATE LEVEL AND INCREASING POLYPEPTIDE PRODUCTION BY DOWNREGULATING THE EXPRESSION OF LACTATE DEHYDROGENASE AND PYRUVATE DEHYDROGENASE KINASE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of International Patent Application No. PCT/US2011/038191, filed May 26, 2011; which claims priority benefit to U.S. Provisional Patent Application No. 61/349,727 filed May 28, 2010, the content of each of which is hereby incorporated herein by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing submitted via EFS-Web and hereby incorporated by reference in its entirety. Said ASCII copy, created on Nov. 15, 2012, is named 146392007800, and is 3.83 bytes in size.

FIELD OF THE INVENTION

The field of this invention relates generally to methods and compositions for reducing lactate production and increasing polypeptide production in cultured cells.

BACKGROUND OF THE INVENTION

Biopharmaceutical market is growing rapidly, and the industry is projected to reach $70 billion dollars by year 2010. See Genetic Engineering in Livestock: New Applications and Interdisciplinary Perspectives (Engelhard et al., 2009) Springer Berlin Heidelberg. Given the increase in demand in therapeutic proteins and the increase in competitions in market sharing among companies, there is a need in improving technologies to achieve better productivity in therapeutic proteins. Towards this goal, different approaches, such as host cell engineering, have been explored. See Kuystermans et al., *Cytotechnology* 53(1-3): 3-22 (2007); and O'Callaghan and James, *Brief Funct. Genomic Proteomic* 7(2):95-110 (2008). Cultured cells, such as Chinese Hamster Ovary (CHO) cells, are widely used to produce therapeutic proteins. For example, pH-controlled fed-batch bioreactor culture has been used widely to produce recombinant monoclonal antibodies. Langheinrich and Nienow, *Biotechnol. Bioeng.* 66(3):171-9 (1999). Lactate is one of the main accumulated waste products during fed-batch culture, and it has been shown to inhibit cell growth and protein production. See Glacken et al., *Biotechnol. Bioeng.* 32:491-506 (1988); and Lao and Toth, *Biotechnol. Prog.* 13:688-691 (1997). This in turn leads to an increase in the amount of alkali needed for adding into the culture medium to control the pH. Dietl et al., *J. Immunol.* 184(3):1200-9 (2010); Langheinrich and Nienow, *Biotechnol. Bioeng,* 66(3):171-9 (1999). Increased addition of alkali to the cell culture medium for maintaining the pH can result in an increase in osmolality, and this increase can lead to cell growth inhibition and decreased antibody productivity. Cruz et al., *Enzyme Microb. Technol.* 27(1-2):43-52 (2000); Iran et al., *Biotechnol. Bioeng.* 66:238-246 (1999). Hence, reducing the lactate level is desired for the development of polypeptide or a higher titer antibody production process.

There are many factors that can influence lactate production in cell culture, such as controlling the pyruvate level.

See Liu et al., *J. Biol. Chem.,* 284(5):2811-22 (2009); and Samuvel et al., *J. of Immunol.* 182(4):2476-84 (2009). Pyruvate is the substrate for the enzymes pyruvate dehydrogenase (PDH) and lactate dehydrogenase (LDH).

The PDH complex is a multi-enzyme unit consisting of three catalytic enzymes, E1, E2, and E3. Patel and Korotchkina, *Exp. Mol. Med.* 33(4):191-7 (2001). This complex catalyzes the rate-limiting conversion reaction in converting from pyruvate to acetyl-CoA, which is the entry point of tricarboxylic acid (TCA) cycle. The activity of PDH is regulated by the enzymes pyruvate dehydrogenase kinases (PDHK(s)) and pyruvate dehydrogenase phosphatases (PDHPs). PDHKs phosphorylate PDH to suppress its enzymatic activity, whereas PDHP dephosphorylate and thus activate PDH. See Patel and Korotchkina, *Exp. Mol. Med.* 33(4):191-7 (2001); Roche and Hiromasa, *Cell Mol. Life. Sci.* 64(7-8):830-49 (2007); Holness and Sugden, *Biochemical Society Transactions,* 31:1143-1151 (2003). There are four isotypes of PDHK in mammalian cells (PDHK1, PDHK2, PDHK3, and PDHK4) with tissue specific distributions. See Harris et al., *Adv. Enzyme Regul.* 42:249-59 (2002); and Bowker-Kinley et al., *Biochem. J.* 329(1):191-6 (1998).

LDH directly catalyzes the interconversion of pyruvate and lactate with concurrent interconversion of NADH and NAD+. In mammalian cells, LDHs exist as either homo- or heterotetramers consisting mostly A and B subunits (or H and M subunits, respectively) encoded by LDHa and LDHb genes, and sometimes homotetramers of C subunit encoded by LDHc genes. See Baumgart et al., *J. Biol. Chem.* 271 (7):3846-55 (1996); Li et al., *J. Biol. Chem.* 258(11):7029-32 (1983); Skory C. D., *Appl. Environ. Microbiol.* 66(6): 2343-8 (2000); and Read et al., *Proteins* 43(2):175-185 (2001). For example, in CHO cells, LDH isotypes have been shown to be intermediates of the A3B and A2B2 tetramer. Jeong et al., *Biochem. Biophys. Res. Commun.* 289(5): 1141-9 (2001). Previous studies have shown that downregulating LDHa in CHO cells by disrupting the gene via homologous recombination (Chen et al., *Biotechnol. Bioeng.* 72(1):55-61 (2001)), antisense technology (Jeong et al., *Biochem. Biophys. Res. Commun.* 289(5):1141-9 (2001)), or small or short interfering RNA (siRNA) (Kim and Lee, *Appl. Microbiol. Biotechnol.* 74(1):152-9 (2007)) can reduce lactate level, but did not achieve appreciable improvement in protein productivity. For example, in the case of LDHa specific siRNA, even though there was reportedly a 45-79% reduction in lactate level, there was no significant improvement in Specific Productivity (Qp) and product (antibody) titer, suggesting that knocking down LDHa alone in CHO cells is not sufficient to improve Qp and product yield efficiently. Thus, more efficient methods for reducing lactate production are needed for achieving a better therapeutic polypeptide production.

All publications, patents, and patent applications cited herein are hereby incorporated by reference herein in their entirety for all purposes to the same extent as if each individual publication, patent, and patent application were specifically and individually indicated to be so incorporated by reference.

BRIEF SUMMARY OF THE INVENTION

The present invention provides methods and compositions for reducing lactate production and increasing polypeptide production in cultured cells. The inventors have discovered that concomitant downregulation of a LDH and PDHKs via siRNAs in cultured cells expressing polypep-